S100A9—clot not, bleed not

By Michael J. Haas, Senior Writer

Although the new generation of antithrombotic drugs provides marked improvements over warfarin, they all still carry bleeding risk. S100 calcium binding protein A9 could represent a new target that, when blocked, prevents thrombosis without increasing that risk.[1]

The new findings from U.S. researchers may hand a new indication to at least two companies—**InflammatoRx Inc.** and **Active Biotech AB**—developing inhibitors of this S100 protein to treat cancer, autoimmune diseases and inflammation.

S100 proteins are a family of signaling molecules whose individual members play multiple roles in many cells and tissue types. S100 calcium binding protein A9 (S100A9; calgranulin B; MRP14) is expressed by neutrophils and monocytes and is activated by endothelial, epithelial and synovial cells.

Although its intracellular functions are poorly understood, secreted S100A9 can stimulate the production of proinflammatory cytokines by monocytes and promote the migration and adhesion of neutrophils and monocytes to sites of inflammation.

S100A9 frequently occurs as a heterodimer with S100A8 (calgranulin A; MRP8), whose function requires S100A9 to bind and stabilize it.

In 2006 and 2008, teams led by Daniel Simon identified an S100A8/S100A9 heterodimer expressed by platelets as a risk marker for acute ST-segment elevation myocardial infarction (STEMI)—a type of heart attack involving abnormal cardiac electrophysiology—in previously healthy individuals.[2] The heterodimer also was a marker for the recurrence of cardiovascular events such as MI, stroke and death in patients with acute coronary syndrome (ACS).[3]

Simon is chief of cardiovascular medicine and director of the Harrington Heart & Vascular Institute at **University Hospitals Case Medical Center** and a professor of cardiovascular research at **Case Western Reserve University School of Medicine**.

In 2009, another Simon-led team showed that S100A9 regulates vascular inflammation and responses to vascular injury in mouse models of atherosclerosis, vascular inflammation and restenosis by promoting leukocyte recruitment to vascular lesions.[4]

For the new study, Simon's team set out to determine whether platelet-expressed S100A8/S100A9 played a causal role in arterial thrombosis and thus might be a therapeutic target to prevent or treat thrombotic events.

In wild-type mouse models of arterial thrombosis, S100A8/S100A9 expression in platelets was upregulated and platelets secreted S100A8/A100A9 in response to clot-stimulating thrombin (factor IIa; F2). Also in the models, *S100a9* deficiency decreased thrombin-induced platelet activation and accumulation on arterial walls, thereby increasing the time to thrombus formation, compared with unmodified *S100a9* expression.

Moreover, there was no difference in tail vein bleeding time—a measure of hemostasis and bleeding risk—between the *S100a9* knockout and wild-type mice.

Next, the team conducted thrombosis assays in whole blood from the *S100a9*-deficient mice and normal human blood pretreated with a research antibody against S100A9. The time to thrombus formation was longer than that for blood from wild-type mice or human blood pretreated with an inactive control antibody.

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> ***—Daniel Simon, Case Western Reserve University School of Medicine***

Additional experiments in mice confirmed that signaling between S100a9 secreted specifically by platelets and Cd36 (Gpiv) on other platelets drove thrombosis in the models.

Lastly, the team found platelet-derived S100A9 in arterial thrombi from patients with STEMI—a finding that further supported a causal role for S100A9 in thrombotic events in patients.

"Our paper shows that you can inhibit arterial thrombosis in mice without prolonging bleeding time or affecting parameters that influence hemostasis" by blocking S100A9, Simon told *SciBX*. "Targeting S100A9 would thus be useful in preventing and/or treating thrombosis in patients" with ACS, stroke and other conditions involving thrombosis.

Simon's team included researchers from **Brigham and Women's Hospital**, **Harvard Medical School**, the **Medical College of Wisconsin**, **Cancer Research UK**'s **London Research Institute** and **Portola Pharmaceuticals Inc.**, which conducted the thrombosis assays in human blood.

Data were reported in *The Journal of Clinical Investigation*.

Francesc Mitjans, CSO of **Lykera Biomed S.A.**, said, "Inhibiting S100A9 appears to be a potential strategy to prevent or treat thrombosis."

S100 possibilities

Mitjans said that the study raises the question of whether other members of the S100 family of proteins—some of which have been implicated in atherosclerosis[5] and cardiac fibrosis[6]—might also be involved in thrombotic processes.

Thus, he wanted to see head-to-head comparisons between inhibitors of different S100 proteins and whether the targets synergize with approved antithrombotic drugs.

Marketed antithrombotic therapies include inhibitors of factor Xa, inhibitors of thrombin and formulations of low molecular weight heparin (LMWH) that inhibit both factors Xa and IIa. All of the therapies have better safety profiles than warfarin and other coumarins—which inhibit vitamin K–dependent synthesis of multiple clotting factors and the proteins that regulate them—but still carry a risk of GI bleeding.

Philippe Tessier, president and CSO of InflammatoRx, was less sanguine about the prospects of inhibiting S100A9 to prevent or treat thrombosis.

"While this is an interesting study, the antithrombotic effect of blocking S100A9 does not seem very drastic, so I do not see an anti-S100A9 therapy as a first-line treatment in cardiovascular patients at high risk of thrombosis," he said.

Instead, he said, anti-S100A9 therapy might help prevent thrombosis in a different patient population—those with autoimmune diseases.

"High concentrations of S100A9 are found in the serum and at sites of inflammation in patients with diseases like rheumatoid arthritis, psoriasis and Crohn's disease, and we know that patients with autoimmune diseases are at risk for cardiovascular disease," he said. "The *JCI* study indicates that the presence of S100A9 in serum might enhance thrombosis and thus explain the risk of thrombotic events" in patients with these autoimmune diseases.

"The study suggests that targeting S100A9 to reduce inflammation in patients with autoimmune disease might have the added benefit of preventing thrombosis."
—Philippe Tessier, InflammatoRx Inc.

In turn, "the study suggests that targeting S100A9 to reduce inflammation in patients with autoimmune disease might have the added benefit of preventing thrombosis," he said.

InflammatoRx has a humanized antibody against S100A9 in preclinical development to treat undisclosed inflammatory indications.

Tessier said that the *JCI* study "indicates that our antibody might reduce both inflammation and the risk of thrombosis in patients with autoimmune disease" and thus might differentiate InflammatoRx's product from other autoimmune therapies.

Mitjans and Tessier agreed that inhibiting S100A9 to prevent or treat thrombosis would probably have few—if any—side effects.

"Under normal physiological conditions, there are no reports of secreted S100 proteins, indicating they maintain only intracellular roles," Mitjans said. "S100 proteins appear to be secreted and released into the extracellular milieu only under pathological conditions."

Tessier said that unpublished preclinical safety studies by InflammatoRx found that prolonged treatment with an anti-S100A9 antibody had no obvious side effects in mice, and "mice deficient in *S100a9* or treated with the antibody can still develop normal immune responses during infection."

Thus, an anti-S100A9 antibody could have the desired antithrombotic effect without blocking the protein's intracellular functions, he said. "But, of course, there are a few instances—such as wound healing and menstruation—where thrombus formation is part of the normal process," and the safety of anti-S100A9 therapy would have to be closely scrutinized.

Tessier said that an antibody against S100A9 would be preferable to a small molecule inhibitor because S100A9 binds to multiple receptors—among them CD36, receptor for advanced glycation endproducts (RAGE) and toll-like receptor 4 (TLR4). A small molecule would be unlikely to block S100A9's interactions with all of them.

Lykera has three antibodies against S100 proteins in preclinical development to treat solid tumors: LK-1, a humanized mAb against S100A4; LK-3, a humanized mAb against S100 calcium binding protein P (S100P); and LK-5, a mAb against S100A7 (psoriasin).

Knockout technicality

A significant gap in the *JCI* study was that the team only tested the antithrombotic effects of *S100a9* knockout—not an actual S100A9 inhibitor—in the mouse models.

Although the team was unable to test its anti-S100A9 antibody in the models because it did not target the mouse protein, "there are other possible options, such as tasquinimod" for testing S100A9 inhibition *in vivo*, said Mitjans.

Active Biotech and **Ipsen Group** have tasquinimod (ABR-215050; TASQ), an oral quinoline-3-carboxamide derivative that binds S100A9, in Phase III testing to treat prostate cancer and in Phase II testing to treat gastric, liver, ovarian and renal cancers.

In addition, Active Biotech and **Teva Pharmaceutical Industries Ltd.** have Nerventra laquinimod, an oral quinoline-3-carboxamide immunomodulator that targets S100A9, in registration to treat multiple sclerosis (MS) and in Phase II testing to treat Crohn's disease and lupus. Active Biotech also has the compound in Phase II testing to treat Huntington's disease (HD).

Active Biotech's paquinimod (ABR-215757), a small molecule quinoline-3-carboxamide immunomodulator that targets S100A9, is in Phase II testing to treat lupus.

Simon said that his team had not yet contacted Active Biotech but was thinking about doing so. Active Biotech did not respond to requests for comment.

Simon's team is now investigating whether levels of S100A8/S100A9 in platelets can predict differing risks of coronary artery disease (CAD) events in patients with ACS versus those with stable CAD.

"We have preliminary data showing that platelet expression of the heterodimer is increased in ACS compared with CAD," suggesting that S100A8/S100A9 levels could help identify patients with ACS in whom anti-S100A9 or anti-platelet therapy might reduce the risk of a cardiovascular event, Simon said. This is an important finding because "we currently have no markers to predict the risk of plaque rupture and future myocardial infarction or stroke."

The team is also investigating the role of S100A9 in venous thrombosis and—with collaborators at the **University of Michigan**—conducting genomewide association studies to identify the genes involved in regulating plasma levels of S100A8/S100A9.

Simon noted that although levels of the heterodimer predict first and recurrent heart attacks, the team's studies in arterial thrombi from patients with STEMI showed that not all platelets expressed S100A8/S100A9, for reasons that were unclear.

However, "we know from preliminary data that plasma levels of S100A8/S100A9 are inheritable—meaning that approximately 40% of the variability in those levels is genetically controlled," he said. "The genomewide studies will give us insights into which genes are responsible for this variability" and might reveal potential antithrombotic targets upstream of S100A9.

He added that it was unlikely that those upstream targets would have the same therapeutic potential as S100A9 because the prothrombotic role of S100A9-CD36 signaling between platelets is distinct from the pro-clotting role of platelets in response to injury.

According to Simon, **Case Western Reserve University** has patented the findings reported in *JCI*, and the IP is unlicensed.

Haas, M.*J. SciBX* 7(21); doi:10.1038/scibx.2014.605
Published online May 29, 2014

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